

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : <b>A61K 37/36</b>	<b>A1</b>	(11) International Publication Number: <b>WO 91/19513</b> (43) International Publication Date: 26 December 1991 (26.12.91)
(21) International Application Number: PCT/US91/04449 (22) International Filing Date: 20 June 1991 (20.06.91) (30) Priority data: 541,221 20 June 1990 (20.06.90) US (71) Applicant: BRISTOL-MYERS SQUIBB COMPANY [US/US]; 345 Park Avenue, New York, NY 10154 (US). (72) Inventors: OLESON, Frederick, B., Jr. ; 5104 Shiraz Lane, Fayetteville, NY 13066 (US). COMERESKI, Charles, R. ; 510 David Drive, North Syracuse, NY 13088 (US). (74) Agent: POOR, Brian, W.; Bristol-Myers Squibb Company, 3005 First Avenue, Seattle, WA 98121 (US).	(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent).  Published <i>With international search report.</i>	

(54) Title: METHODS OF MODULATING BLOOD PRESSURE USING TGF- $\beta$  AND ANTAGONISTS THEREOF

(57) Abstract

The use of TGF- $\beta$  and TGF- $\beta$  antagonists to modulate blood pressure is described. In a specific embodiment described by way of example herein, recombinant mature TGF- $\beta$ 1 isolated and purified from transfected Chinese Hamster Ovary cells induced rapid, significant and sustained decreases in arterial blood pressure of cynomolgus monkeys receiving daily injections of the rTGF- $\beta$ 1. The TGF- $\beta$  used to lower blood pressure may be obtained from native sources or may be produced by recombinant DNA or chemical synthetic techniques.

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METHODS OF MODULATING BLOOD  
PRESSURE USING TGF- $\beta$  AND ANTAGONISTS THEREOF

1. INTRODUCTION

The present invention is directed to the use of transforming growth factor-beta (TGF- $\beta$ ) and TGF- $\beta$  antagonists to modulate blood pressure (BP). The method of the invention is demonstrated by way of example in which mature recombinant TGF- $\beta$ 1 (rTGF- $\beta$ ) is used to rapidly lower blood pressure in adult cynomolgus monkeys. However, the scope of the invention is not limited to the use of rTGF- $\beta$ 1 but rather encompasses the use of mature and precursor forms of all members of the TGF- $\beta$  family effective at modulating blood pressure, including natural and recombinant mature TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, TGF- $\beta$ 4, etc., as well as TGF- $\beta$  hybrids, analogs and latent TGF- $\beta$  complexes. Similarly, the invention includes the use of any and all compositions effective at antagonizing TGF- $\beta$  activity, including but not limited to anti-TGF- $\beta$  antibodies and TGF- $\beta$  receptors.

2. BACKGROUND OF THE INVENTION

2.1. TRANSFORMING GROWTH FACTOR-BETA

TGF- $\beta$  is a member of a recently described family of polypeptides that regulate cellular differentiation and proliferation. Other members of this family include Mullerian inhibitory substance (Cate et al., 1986, Cell 45:685-698), the inhibins (Mason et al., 1985, Nature 318:659-663) and a protein predicted from a transcript of the decapentaplegic gene complex of *Drosophila* (Padgett et al., 1987, Nature 325: 81-84).

Four types of TGF- $\beta$  have been identified and designated TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 1.2, and TGF- $\beta$ 3. The first described type, TGF- $\beta$ 1, consists of two identical disulfide linked subunits having molecular weights of 13,000 (Assoian et al., 1983, J. Biol. Chem. 258:7155-7160; Frolik et al.,

1983, Proc. Natl. Acad. Sci. USA 80:3676-3680; Frolik et al., 1984, J. Biol. Chem. 260:10995-11000). It has been purified from several tissue sources including placenta (Frolik et al., 1983, Nature 325:81-84), blood platelets (Childs et al., 1982, Proc. Natl. Acad. Sci. USA 79:5312-5316; Assoian et al., 1983, J. Biol. Chem. 258:7155-7160) kidney (Roberts et al., 1983, Biochemistry 22:5692-5698), and demineralized bone (Seyedin et al., 1985, Proc. Natl. Acad. Sci. USA 82:119-123). cDNA clones coding for human (Derynck et al., 1985, Nature 316:701-705), mouse (Derynck et al., 1986, J. Biol. Chem. 261:4377-4379) and simian (Sharples et al., 1987, DNA 6:239-244) TGF- $\beta$ 1 have been isolated. DNA sequence analysis of these clones indicates that TGF- $\beta$ 1 is synthesized as a large precursor polypeptide, the carboxy terminus of which is cleaved to yield the mature TGF- $\beta$  monomer. Strong sequence homology has been found throughout the TGF- $\beta$ 1 precursor protein from all of the above sources.

In the presence of 10% serum and epidermal growth factor, TGF- $\beta$ 1 promotes the anchorage independent growth of normal rat kidney fibroblasts (Roberts et al., 1981, Proc. Natl. Acad. Sci. USA 78:5339-5343; Roberts et al., 1982, Nature 295:417-419; Twardzik et al., 1985, J. Cell. Biochem. 28:289-297); in the presence of 10% serum alone, it is able to induce colony formation of AKR-2B fibroblasts (Tucker et al., 1983, Cancer Res. 43:1518-1586). TGF- $\beta$ 1 has also been shown to cause fetal rat muscle mesenchymal cells to differentiate and produce cartilage specific macromolecules (Seyedin et al., 1986, J. Biol. Chem. 261:5693-5695).

In contrast to its effect on cell proliferation, TGF- $\beta$ 1 purified from human platelets has been shown to inhibit the growth of certain cells in culture (Tucker et al., 1984, Science 226:705-707). TGF- $\beta$ 1 has also been shown to inhibit the growth of several human cancer cell lines

(Roberts et al., 1985, Proc. Natl. Acad. Sci. USA 82:119-123). This inhibitory/stimulatory effect of TGF- $\beta$ 1 may depend on several factors including cell type and the physiological state of the cells (for review see Sporn et al., 1986, Science 233:532-534).

TGF- $\beta$ 2, like TGF- $\beta$ 1, is a polypeptide of molecular weight 26,000 composed of two identical 13,000-dalton subunits which are disulfide like (Chiefetz et al., 1987, Cell 48:409-415; Ikeda et al., 1987, Biochemistry 26:2406-2410) and has been isolated from bovine demineralized bone (Seydin et al., 1987, J. Biol. Chem. 262:1946-1949), porcine platelets Chiefetz et al., 1987, 48:409-415), a human prostatic adenocarcinoma cell line, PC-3 (Ikeda et al., 1987, biochemistry 26:2406-2410), and a human glioblastoma cell line (Wrann et al., 1987, EMBO 6:1633-1636). cDNA clones coding for human and simian TGF- $\beta$ 2 have been isolated (Madisen et al., 1988, DNA 7:1-8; Webb et al., 1988, DNA 7:493-497). The mature TGF- $\beta$ 2 monomer is cleaved from one of two larger precursor polypeptides, the mRNAs of which may arise via differential splicing (Webb et al., 1988, DNA 7:493-497).

TGF- $\beta$ 1 and TGF- $\beta$ 2 share 71% amino acid sequence identity in their mature regions, and 41% identity in their precursor structures. TGF- $\beta$ 3, the amino acid sequence of which has very recently been deduced from cDNA clones, appears to contains C-terminal 112 amino acid sequence with about 80% homology to the mature monomers of TGF- $\beta$ 1 and TGF- $\beta$ 2 (Dijke et al., 1988, Proc. Natl. Acad. Sci. USA 85:4715-4719). TGF- $\beta$ 1.2 is a heterodimeric form comprising a  $\beta$ 1 and  $\beta$ 2 subunit linked by disulfide bonds (Chiefetz et al., 1987, Cell 48:409-415).

### 3. SUMMARY OF THE INVENTION

The present invention is directed to methods of modulating blood pressure using TGF- $\beta$  polypeptides, TGF- $\beta$  antagonists, and/or combinations thereof. The invention may be subdivided into two categories solely for the purpose of description.

First, the invention relates to the use of TGF- $\beta$ s as antihypertensive agents capable of rapidly and significantly lowering blood pressure. This aspect of the invention encompasses the use of any and all TGF- $\beta$  polypeptides having a hypotensive activity, including mature and precursor forms of TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, hybrid TGF- $\beta$ s, latent TGF- $\beta$  complexes, TGF- $\beta$  analogs, etc. In a specific embodiment of the invention, described more fully by way of example herein (Section 6., *infra*), simian recombinant TGF- $\beta$ 1 is administered parenterally to induce rapid significant, and sustained decreases of arterial blood pressure in cynomolgus monkeys. In a related embodiment, TGF- $\beta$ s may be used to rapidly lower blood pressure to normal levels in patients facing acute hypertension and emergency conditions associated with extreme hypertension.

Second, the invention relates to the use of TGF- $\beta$  antagonists to elevate blood pressure through the inhibition of hypotension induced by TGF- $\beta$  and/or related factors. Any composition which antagonizes TGF- $\beta$  activity may be useful in this regard, including for example, anti-TGF- $\beta$  antibodies and TGF- $\beta$  receptors. Additionally, methods which lower and/or maintain the level of circulating TGF- $\beta$  in an individual may result in a similar pressor effect. For example, anti-TGF- $\beta$  antisense RNA molecules may inhibit synthesis and release of bioactive TGF- $\beta$ s, thereby preventing excessive hypotensive signal generation and resulting hypotension.

#### 4. DESCRIPTION OF THE FIGURES

FIG. 1. Nucleotide sequence of simian TGF- $\beta$ 1 cDNA and deduced amino acid sequence. The 1600 bp insert of pTGF- $\beta$ 1-2 was subcloned into the M13mp18 and M13mp19 cloning vectors (Yanisch-Perron et al., 1985, Gene 33:103-119) and both strands were sequenced using the dideoxy chain-termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci. USA 74:5463-5467). The deduced amino acid sequence of simian TGF- $\beta$ 1 is presented directly above the cDNA sequence. The human TGF- $\beta$ 1 nucleotide sequence is aligned with and presented directly below the simian cDNA sequence; dots indicate homologous nucleotide residues within the sequences. Amino acid differences between the human and simian proteins are indicated in the top line. The mature TGF- $\beta$ 1 sequence is boxed and the signal peptide is overlined.

FIG. 2. Nucleotide sequence of human TGF- $\beta$ 2-442 cDNA and deduced amino acid sequence. The 2597 BP insert of PC-21 was subcloned into pEMBL (Dante et al., 1983, Nucleic Acids Res. 11:1645-1654) and sequenced on both strands using the dideoxy chain-termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci. USA 74:5463-5467). The coding sequence is shown and the deduced amino acid sequence is presented directly above. The mature TGF- $\beta$ 2 sequence is boxed and the signal peptide is overlined. Potential glycosylation sites are indicated by asterisks. The arrow indicates the putative signal sequence cleavage site. The nucleotide sequence of simian TGF- $\beta$ 2-414 cDNA is identical to the human TGF- $\beta$ 2-442 cDNA sequence except that (a) nucleotides 346 through 432 (bracketed) are deleted and replaced by the sequence AAT, and (b) several silent nucleotide changes occur elsewhere in the structure (indicated by single letters directly below the changed nucleotide). The deduced amino acid sequence for simian

TGF- $\beta$ 2-414 precursor is identical to the human TGF- $\beta$ 2-442 precursor amino acid sequence except that Asparagine replaces amino acid residues 116 through 144 in the human TGF- $\beta$ 2-442 structure. The nucleotide sequence of a human TGF- $\beta$ 2-414 cDNA has been sequenced through the region indicated by broken underlining and was found to be perfectly homologous to the human TGF- $\beta$ 2-442 cDNA sequence except that nucleotides 346 through 432 are deleted and replaced by the sequence AAT.

FIG. 3. Nucleotide sequence of hybrid TGF- $\beta$ 1/ $\beta$ 2 precursor DNA and deduced amino acid sequence. The coding sequence is shown and the deduced amino acid sequence is presented directly above. The mature TGF- $\beta$ 2 sequence is boxed and the precursor signal peptide is overlined. Glycosylation sites are indicated by asterisks. The arrow indicates the putative signal sequence cleavage site. The TGF- $\beta$ 2 mature coding sequence depicted is of human origin. The simian TGF- $\beta$ 2 mature coding sequence is nearly identical to the human sequence: only 3 silent base changes occur and are indicated by single letters directly below the changed nucleotide.

##### 5. DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to methods of modulating blood pressure in an animal using TGF- $\beta$  polypeptides, antagonists and/or combinations thereof. The invention is based upon the discovery that parenterally administered mature rTGF- $\beta$ 1 rapidly and significantly lowers blood pressure in cynomolgus monkeys. Thus, one aspect of the invention relates to the use of TGF- $\beta$ s as antihypertensive/hypotensive agents. Precisely the opposite effect, i.e., raising and/or maintaining blood pressure, may be achieved by TGF- $\beta$  antagonists capable of inhibiting the antihypertensive/hypotensive effects of TGF- $\beta$ . In this



regard, the invention encompasses the use of anti-TGF- $\beta$  antibodies, TGF- $\beta$  receptors and other compositions capable of inhibiting TGF- $\beta$ -induced hypotension.

#### 5.1. USE OF TGF- $\beta$ s AS ANTIHYPERTENSIVE AGENTS

One aspect of the invention relates to the use of TGF- $\beta$ s as antihypertensive/hypotensive agents. Applicants' initial data indicates that rTGF- $\beta$ 1 can rapidly and significantly lower blood pressure in simian test subjects at a dosage which appears to be at or close to the physiologically tolerable limit. In this regard, parenteral administration of a TGF- $\beta$  at such a dose may be acceptable in hypertensive emergencies requiring aggressive treatment. Lower doses of a TGF- $\beta$  may also be effective at reducing blood pressure, and such doses may be appropriate for patients with moderate to severe hypertension. In these patients, less aggressive therapy may be desirable where adverse side effects can not be tolerated.

Human patients with diastolic blood pressure greater than 130 mm Hg and complications such as hypertensive encephalopathy, progressive renal failure, acute pulmonary edema, cerebral accident, papilledema, or multiple fresh retinal hemorrhages are generally treated aggressively with a parenteral antihypertensive agent such as, for example, nitroprusside and diazoxide. Treatment of hypertension characterized by such acute complications generally aims to lower BP to about 100 mm Hg within 30 to 60 minutes, since rapid decrease is a key determinant of survival in patients facing these emergencies.

The TGF- $\beta$  antihypertensive may be administered alone or in combination with other antihypertensive agents in suitable pharmacological carriers via any appropriate route. In hypertensive emergencies, parenteral administration will provide the fastest decrease in BP and

is therefore the recommended route of administration in such situations. Additionally, the TGF- $\beta$  may be linked to a carrier or targeting molecule and/or incorporated into liposomes, microcapsules, and controlled release preparations prior to administration in vivo.

#### 5.1.1. SOURCES OF TGF- $\beta$

In accordance with the invention, mature and/or precursor forms of TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, TGF- $\beta$ 1/ $\beta$ 2, etc., may be used to lower blood pressure. The TGF- $\beta$  used may be obtained from a variety of sources, including but not limited to isolating natural TGF- $\beta$ s from appropriate sources, producing TGF- $\beta$  by recombinant DNA techniques, or by chemical synthetic methods, etc.

##### 5.1.1.1. TGF- $\beta$ 1

Natural TGF- $\beta$ 1 can be isolated from a variety of sources. This potent modulator of cell behavior is synthesized by a variety of normal and transformed cells in culture (Roberts et al., 1981, Proc. Natl. Acad. Sci. USA 78:5339-5343) and has been purified from various sources including placenta (Frolik et al., 1983, Proc. Natl. Acad. Sci. USA 80:3676-3680), kidney (Roberts et al., 1983, Biochemistry 22:5692-5698), urine (Twardzik et al., 1985, J. Cell. Biochem. 28:289-297) and blood platelets (Childs et al., 1982, Proc. Natl. Acad. Sci. USA 79:5312-5316). Additionally, the human (Derynck et al., 1985, Nature 316:701-705), mouse (Derynck et al., 1986, J. Biol. Chem. 261:4377-4379), and simian (Sharples et al., 1987, DNA 6:239-244) TGF- $\beta$ 1 have been described.

Large quantities of TGF- $\beta$ 1 may be obtained by recombinant DNA techniques using eucaryotic host cells transfected with recombinant DNA vectors containing the

TGF- $\beta$ 1 coding sequence controlled by expression regulatory elements. Examples of such methods are described in copending application Serial No. 07/353,728 filed August 17, 1989, which application is incorporated by reference herein in its entirety. Briefly, a cDNA clone coding for simian TGF- $\beta$ 1 precursor was obtained from a cDNA library made from an African Green Monkey cell line, BSC-40. The deduced amino acid sequence of the mature simian TGF- $\beta$ 1 shown in FIG. 1 has 100% homology with that of the mature human TGF- $\beta$ 1. Expression vectors were constructed which contain the entire coding sequence for the simian TGF- $\beta$ 1 precursor placed under the control of SV40 expression elements. They were used to transfect Chinese Hamster Ovary cells (CHO cells). The resulting CHO transfectants produce and secrete primarily a high molecular weight complex from which mature bioactive TGF- $\beta$  may be liberated by a routine acidification procedure.

#### 5.1.1.2. TGF- $\beta$ 2

Natural TGF- $\beta$ 2 used in accordance with the invention can be obtained from a variety of sources. A protein isolated from bovine demineralized bone has been identified as being related to TGF- $\beta$  (Seyedin et al., 1987, J. Biol. Chem. 262:1946-1949). The protein has also been isolated from porcine platelets (Cheifetz et al., 1987, Cell 48:409-415), a human prostatic adenocarcinoma cell line PC-3 (Ikeda et al., 1987, Biochemistry 26:2406-2410), and a human glioblastoma cell line (Wrann et al., 1987, EMBO 6:1633-1636). Partial amino acid sequence of this protein indicated that it was homologous to TGF- $\beta$  and has been termed TGF- $\beta$ 2.

Large quantities of TGF- $\beta$ 2 may be obtained by recombinant DNA techniques using eukaryotic host cells transfected with recombinant DNA vectors containing a TGF- $\beta$ 2 coding sequence controlled by expression regulatory

elements. Examples of such methods are described in copending application Serial No. 07/446,020 filed December 5, 1989, which application is incorporated by reference herein in its entirety. Briefly, cDNA clones coding for human TGF- $\beta$ 2 precursor were obtained from a cDNA library made from a tamoxifen treated human prostatic adenocarcinoma cell line, PC-3. The cDNA sequence of one such clone is shown in FIG. 2 and predicts that TGF- $\beta$ 2 is synthesized as a 442 amino acid polypeptide precursor from which the mature 112 amino acid TGF- $\beta$ 2 subunit is derived by proteolytic cleavage. This TGF- $\beta$ 2 precursor, termed TGF- $\beta$ 2-442, shares a 41% homology with the precursor of TGF- $\beta$ 1. In another embodiment, cDNA clones coding for simian TGF- $\beta$ 2 precursor were obtained from a cDNA library made from an African green monkey kidney cell line, BCS-40. The cDNA sequence of one such clone predicts that TGF- $\beta$ 2 is also synthesized as a 414 amino acid polypeptide precursor from which the mature 112 amino acid TGF- $\beta$ 2 subunit is derived by proteolytic cleavage. This TGF- $\beta$ 2 precursor, termed TGF- $\beta$ 2-414, has an amino acid sequence of 414 amino acid residues and is identical to the amino acid sequence of TGF- $\beta$ 2-442, except that it contains a single Asparagine residue instead of the 29 amino acid sequence from residue numbers 116 to 135 of the human TGF- $\beta$ 2-442 sequence.

Clones from the BSC-40 cDNA library which encode a simian TGF- $\beta$ 2-442 precursor as well as clones from the human PC-3 cDNA library which encode a human TGF- $\beta$ 2-414 precursor have also been identified. The human and simian TGF- $\beta$ 2-442 precursors appear to be perfectly homologous at the amino acid level, as do the human and simian TGF- $\beta$ 2-414 precursors.

#### 5.1.1.3. HYBRID MATURE AND PRECURSOR TGF- $\beta$ s

Hybrid mature TGF- $\beta$  molecules may be prepared using recombinant DNA techniques or synthetic methods. Examples of such methods are also described in copending applications Serial No. 284,972, filed December 15, 1988, which application is incorporated by reference herein in its entirety.

Hybrid precursor TGF- $\beta$  molecules may be prepared using recombinant DNA techniques or synthetic methods, as described in co-pending application Serial No. 07/353,728 filed August 17, 1987. Briefly, expression vectors containing the TGF- $\beta$ 2 mature coding sequence joined in-phase (i.e., in the same translational reading frame) to the TGF- $\beta$ 1 signal and precursor sequences (see FIG. 3) were constructed and used to transfect Chinese Hamster Ovary cells (CHO cells). The resulting CHO transfectants produce and secrete mature, biologically active TGF- $\beta$ 2.

#### 5.1.1.4. MODIFIED TGF- $\beta$

Variations in the amino acid sequences shown herein for the different TGF- $\beta$  molecules, as well as variations in the steric configuration, the type of covalent bonds which link the amino acid residues, and/or addition of groups to the amino- or carboxy-terminal residues are within the scope of the invention. For example, the TGF- $\beta$  molecules used in accordance with the invention may include altered sequences such as conservative alterations which result in a silent change thus producing a functionally equivalent molecule. Thus, the amino acid sequences shown in FIGS. 1-3 may be altered by various changes such as insertions, deletions and substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use. As used herein, conservative substitutions would involve the substitution of

one or more amino acids within the sequences shown with another amino acid having similar polarity and hydrophobicity/hydrophilicity characteristics resulting in a silent alteration and a functionally equivalent molecule. Such conservative substitutions include but are not limited to substitutions within the following groups of amino acids: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; phenylalanine, tyrosine; and methionine, norleucine.

#### 5.1.1.5. LATENT TGF- $\beta$ COMPLEX

TGF- $\beta$ 1 may be isolated from tissues or tissue culture cells in an inactive, biologically latent form which may be activated by chaotropic agents, proteases, or in vivo. Similarly, CHO cell transfected with the simian TGF- $\beta$ 1 precursor coding sequence secrete a high molecular weight latent complex involving both the mature and "pro" regions of the TGF- $\beta$  precursor. The association of the "pro" region of the TGF- $\beta$  precursor has also been observed in latent TGF- $\beta$ 1 complex isolated from platelets. Although the mechanism of activation in vivo is unknown, it is possible that the latent complex provides an important level of regulation on TGF- $\beta$ 1 bioactivity. In accordance with the method of the invention, latent TGF- $\beta$  complex may be useful as a means of controlling the hypotensive effect induced by the bioactive form of TGF- $\beta$  by releasing it at the situs of natural in vivo activation mechanisms. The identification, isolation and characterization of latent TGF- $\beta$ 1 complex from recombinant CHO cells is described more fully in copending application Serial No. 07/353,728 filed August 17, 1989, which application is incorporated herein by reference in its entirety.

## 5.2. USE OF TGF- $\beta$ ANTAGONISTS AS PRESSOR AGENTS

Applicants' discovery that rTGF- $\beta$ 1 is capable of rapidly and significantly lowering blood pressure suggests that the TGF- $\beta$ s may be involved in the regulation of BP and/or in the genesis of hypotension. In this regard, through their ability to impede the hypotensive effect of TGF- $\beta$ , antagonists of TGF- $\beta$ s may be useful as pressor/hypotensor agents capable of elevating BP. Any composition which effectively antagonizes the hypotensive effect of a TGF- $\beta$  may be used for this purpose, including but not limited to anti-TGF- $\beta$  antibodies and TGF- $\beta$  receptors.

For example, TGF- $\beta$  antagonists may be useful in treating medical conditions characterized by a loss of BP where the elevation of BP to normal levels is desirable. Such conditions include, for example, shock associated with blood volume loss, cardiac emergencies, and hypotension in acute renal failure. The TGF- $\beta$  antagonists may be administered alone or in combination and/or together with other pressors/hypotensors such as dopamine, epinephrine, aminophylline, etc. Compounds containing effective doses of TGF- $\beta$  antagonist formulated in a suitable pharmacological carrier may be administered to patients experiencing hypotension or conditions associated with hypotension via any appropriate route including but not limited to injection, infusion and selective catheterization in order to elevate BP. In addition, the TGF- $\beta$  antagonist may be linked to a carrier or targeting molecule and/or incorporated into liposomes, microcapsules, and controlled release preparations prior to administration in vivo.

#### 5.2.1. ANTI-TGF- $\beta$ ANTIBODIES

Antibodies capable of inhibiting the hypotensive effect of TGF- $\beta$  may be useful as pressor agents. Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of TGF- $\beta$ s. For the production of antibodies, various host animals can be immunized by injection with a TGF- $\beta$ , or a synthetic TGF- $\beta$  peptide, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum.

A monoclonal antibody to an epitope of a TGF- $\beta$  can be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256, 495-497), and the more recent human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Antibody fragments which contain the idiotype of the molecule may be generated by known techniques. For example, such fragments include but are not limited to the F(ab')<sub>2</sub> fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub>



fragment, and the two Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

The generation of anti-TGF- $\beta$  antibodies is described in copending application Serial No. 7/353,728 filed August 17, 1989, and in copending application Serial No. 07/446,020 filed December 5, 1989.

#### 5.2.2. TGF- $\beta$ RECEPTORS

Exogenous TGF- $\beta$  receptor molecules may be useful pressor agents inasmuch as they are capable of binding circulating TGF- $\beta$  and/or out-competing endogenous receptors which may initiate the hypotensive effect of TGF- $\beta$ . TGF- $\beta$  receptors may be prepared by the methods described in copending application Serial No. 269,524 filed November 11, 1988, which application is incorporated by reference herein in its entirety.

#### 6. EXAMPLE: EFFECT OF PARENTERALLY ADMINISTERED rTGF- $\beta$ 1 ON BLOOD PRESSURE IN CYNOMOLGUS MONKEYS

Described below is part of a study designed to evaluate the pharmacotoxic effects of rTGF- $\beta$ 1 following daily intravenous infusions to cynomolgus monkeys (Macaca fascicularis). The results described herein indicate that rTGF- $\beta$ 1 has a profound reducing effect on blood pressure.

#### 6.1. PROTOCOL

##### 6.1.1. CYNOMOLGUS MONKEYS

One adult male and two adult female cynomolgus monkeys were monitored for blood pressure changes resulting from daily TGF- $\beta$ 1 treatment. Monkeys were fed commercially available chow daily and were provided water ad libitum. Blood pressures were measured via chronic arterial catheters surgically implanted at least 5 days prior to the initiation of the study. One of the female monkeys (39-181) underwent

general anesthesia and a chronic venous catheter was implanted in the right illiac vein. Approximately one month later, this catheter was removed, and the monkey was aseptically implanted with chronic arterial and venous catheters in the illiac artery and vein. The other female monkey (29-825) and the male monkey (B-344) were also implanted with chronic venous and arterial catheters in the illiac artery and vein. The catheters were exteriorized via a tether apparatus as a venous access to facilitate the administration of the test article or vehicle control.

#### 6.1.2. TEST ARTICLE FORMULATION

The formulation of the test and/or control articles was performed daily prior to administration. Each 10 ml of test article dosing solution (0.0213 mg/ml) was prepared by mixing 0.66 ml of rTGF- $\beta$ 1 stock solution (0.46 mg/ml in 5mM HCl) with 9.34 ml of 0.1% monkey serum albumin in PBS solution. The pH of the dosing solution was recorded after formulation and prior to administration each day. Dose volumes were calculated based on the most recent non-tethered body weights and rounded to the nearest 0.1 ml.

Recombinant mature TGF- $\beta$ 1 was isolated and purified from the supernatants of cultured Chinese Hamster Ovary cells transfected with the complete simian TGF- $\beta$ 1 precursor coding sequence as described in co-pending application Serial No. 07/353,728 filed August 17, 1989, which application is incorporated by reference herein in its entirety.

#### 6.1.3. TREATMENT

Monkey 39-181 received 1% monkey albumin in PBS (vehicle control) at a volume of 8 ml/kg daily for five consecutive days. Monkey 69-168 was treated for five consecutive days at a dose of 0.17 mg/kg and at a

concentration of 0.0213 mg/ml. rTGF- $\beta$ 1 was administered to Monkeys 29-825, 39-181 and B344 at a dose of 0.51 mg/kg and at a concentration of 0.0639 mg/ml. Monkey 29-825 was treated for three consecutive days, monkey 39-181 for three consecutive days, and monkey B-344 for one day. The body weights used to calculate the dosages of either the test article or control were the most recent body weights obtained without the encumbrance of the tether apparatus.

rTGF- $\beta$ 1 or vehicle control was administered intravenously through chronic venous catheters at a rate of 1.60 ml of dosing solution/minute via an infusion pump (Harvard Apparatus), and calibrated according to the standard operating procedures of the test facility. Prior to the administration of the test article and control, catheter patency was maintained via periodic flushing of the catheter with 0.9% sterile saline. The volume, time, and date of administration of rTGF- $\beta$ 1 and control were recorded.

#### 6.1.4. BLOOD PRESSURE MEASUREMENTS

Blood pressure measurements were recorded from monkeys 29-825, 39-181, and B344 via chronic arterial catheters. Blood pressure measurements were recorded for at least one minute prior to and after completion of the administration of the test article. Blood pressure measurements were also recorded as amended or at the discretion of the study director if such measurements were clinically relevant.

#### 6.2. CLINICAL OBSERVATIONS

The monkeys were observed daily over 29 days for clinical abnormalities, food and water intake, body temperature, respiration rate, blood pressure, and other

parameters. Additionally, blood samples were collected from each monkey to provide samples for hematology, serum chemistry and immunological analyses.

6.2.1. EFFECT OF rTGF- $\beta$ 1 ON BP

Two of the three monkeys receiving rTGF- $\beta$ 1 injections experienced immediate, significant and progressive decreases in arterial blood pressure. The third experimental monkey also experienced BP loss, but these results are somewhat more difficult to interpret in view of the extreme hypotension existing in this animal prior to treatment. No significant BP fluctuations were observed in the control monkey. The individual BP observations for the three experimental monkeys are tabulated in TABLE I.

TABLE I

INDIVIDUAL BLOOD PRESSURE PROFILES OF MALE  
AND FEMALE CYNOMOLGUS MONKEYS TREATED WITH TGF- $\beta$ 1

ANIMAL NUMBER	SEX	DOSE mg/kg	STUDY DAY	MEAL ATERIAL BLOOD PRESSURE (mm Hg)
39-181	F	0.51	1 (Pre)	44
			1 (Post)	48
			1 (4 Hr)	50
			2 (Pre)	60
			2 (Post)	32
			2 (4 Hr)	46
			3 (Pre)	40
			3 (Post)	32
			3 (4 Hr)	12
			4	18
			5	32
29-825	F	0.51	1 (Pre)	108
			1 (Post)	80
			1 (4 Hr)	48
			2 (Pre)	76
			2 (Post)	76
			3 (Pre)	20
			4	52
			5	50
B-344	M	0.51	1 (Pre)	104
			1 (Post)	60
			1 (4 Hr)	36
			2	20
			3	32
			4	22
			5	20
			9	36

Monkeys 29-825 and B-344 experienced an immediate (1 hour post-administration) BP reduction of 26% and 42%, respectively. Four hours after treatment, BP had dropped by 55% in monkey 29-825 and by 65% in monkey B-344. These initial drops in BP were sustained in the subsequent treatment days, resulting in hypotension and shock. Monkey 39-181 did not respond to initial TGF- $\beta$ 1 treatment (day 1) with a reduction in BP, possibly because of its pre-existing hypotensive condition. Interestingly, a slight elevation in BP was observed on day 2 prior to treatment, and a sustained decrease in BP was observed thereafter.

In addition to the dramatic decrease in BP and the accompanying shock/hypotension observed in all three treated animals, other observed effects directly attributable to TGF- $\beta$ 1 included hemotopoietic changes (decrease in erythrocytes, lymphocytes and platelets) and immunological compromise (decrease in lymphocyte responsiveness to mitogen). Additionally, all the treated monkeys had no or minimal appetite, and all were inactive during the treatment period. Monkey 29-825 was recumbent on day 4, required fluid therapy on days 4 and 5, and was euthanized on day 5 because of its moribund condition. Monkey 39-181 had darkened blood on days 3 and 4, developed septicemia on day 8, and was euthanized because of its deteriorating condition on day 8. Monkey B-344 appeared normal from days 6 to 29.

The present invention is not to be limited in scope by the cell lines, TGF- $\beta$  molecules and assays exemplified which are intended as but single illustrations of one aspect of the invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled

in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating hypertension comprising administering a TGF- $\beta$  to an individual at a dose effective at lowering blood pressure.
2. The method of claim 1 wherein the TGF- $\beta$  comprises a mature TGF- $\beta$ 1.
3. The method of claim 1 wherein the TGF- $\beta$  comprises a mature TGF- $\beta$ 2.
4. The method of claim 1 wherein the TGF- $\beta$  comprises a mature TGF- $\beta$ 1/ $\beta$ 2 hybrid.
5. The method of claim 1 wherein the TGF- $\beta$  comprises a TGF- $\beta$ 1 precursor.
6. The method of claim 1 wherein the TGF- $\beta$  comprises a TGF- $\beta$ 2 precursor.
7. The method of claim 1 wherein the TGF- $\beta$  comprises a hybrid TGF- $\beta$ 1/TGF- $\beta$ 2 precursor.
8. The method of claim 1 wherein the TGF- $\beta$  comprises a latent TGF- $\beta$ 1 complex.
9. The method of claim 1 wherein the TGF- $\beta$  comprises a latent TGF- $\beta$ 2 complex.
10. A method of lowering blood pressure in a mammal comprising administering TGF- $\beta$  to the mammal in an amount and for a time period effective at inducing the desired hypotensive effect.



11. The method of claim 10 wherein the TGF- $\beta$  comprises a mature TGF- $\beta$ 1.

12. The method of claim 10 wherein the TGF- $\beta$  comprises a mature TGF- $\beta$ 2.

13. The method of claim 10 wherein the TGF- $\beta$  comprises a mature TGF- $\beta$ 1/ $\beta$ 2 hybrid.

14. The method of claim 10 wherein the TGF- $\beta$  comprises a TGF- $\beta$ 1 precursor.

15. The method of claim 10 where in the TGF- $\beta$  comprises a TGF- $\beta$ 2 precursor.

16. The method of claim 10 where in the TGF- $\beta$  comprises a hybrid TGF- $\beta$ 1/TGF- $\beta$ 2 precursor.

17. The method of claim 10 where in the TGF- $\beta$  comprises a latent TGF- $\beta$ 1 complex.

18. The method of claim 10 where in the TGF- $\beta$  comprises a latent TGF- $\beta$ 2 complex.

19. A method of treating hypotension comprising administering to an individual a TGF- $\beta$  antagonist capable of inhibiting the hypotensive activity induced by TGF- $\beta$  at a dose effective at inducing an elevation in blood pressure.

20. The method of claim 19 wherein the TGF- $\beta$  antagonist is an anti-TGF- $\beta$  antibody.

21. The method of claim 19 wherein the TGF- $\beta$  antagonist is a TGF- $\beta$  receptor.

22. The method of claim 19 wherein the TGF- $\beta$  antagonist is TGF- $\beta$ 1 antagonist.

23. The method of claim 22 wherein the TGF- $\beta$ 1 antagonist is an anti-TGF- $\beta$ 1 antibody.

24. The method of claim 22 wherein the TGF- $\beta$  antagonist is a TGF- $\beta$ 1 receptor.

25. The method of claim 19 wherein the TGF- $\beta$  antagonist is a TGF- $\beta$ 2 antagonist.

26. The method of claim 25 wherein the TGF- $\beta$ 2 antagonist is a TGF- $\beta$ 2 antibody.

27. The method of claim 25 wherein the TGF- $\beta$ 2 antagonist is a TGF- $\beta$ 2 receptor.

28. A method of elevating blood pressure comprising administering to a mammal a TGF- $\beta$  antagonist in an amount and for a time period effective at inducing the desired blood pressure increase.

29. The method of claim 28 wherein the TGF- $\beta$  antagonist is an anti-TGF- $\beta$  antibody.

30. The method of claim 28 wherein the TGF- $\beta$  antagonist is a TGF- $\beta$  receptor.

31. The method of 28 wherein the TGF- $\beta$  antagonist is a TGF- $\beta$ 1 antagonist.

32. The method of claim 31 wherein the TGF- $\beta$ 1 antagonist is an anti-TGF- $\beta$ 1 antibody.

33. The method of claim 31 wherein the TGF- $\beta$ 1 antagonist is a TGF- $\beta$ 1 receptor.

34. The method of claim 28 wherein the TGF- $\beta$  antagonist is a TGF- $\beta$ 2 antagonist.

35. The method of claim 34 wherein the TGF- $\beta$ 2 antagonist is a TGF- $\beta$ 2 antibody.

36. The method of claim 34 wherein the TGF- $\beta$ 2 antagonist is a TGF- $\beta$ 2 receptor.

Simian	-261	AGGGGATCTGTGGCAGGTCGGAGA---AAGATC---CGTCT	-227
Human		CTCC..C...CAA...A..CCCT.TTC....C.ACC.AC..	
Simian		CCTGGTACCAGATCTCGCCCATCTAGGTTATTTCCGTGGGATACTGAGACAC	-175
Human		T.....G.....	
Simian		CCCCGGTCCAAGCCTCCCCTCCACCACTGCGCCCTTCTCCCGTAGGA-CCTC	-123
Human		.....G.....	
Simian		AAC TTTCCTCGAGGCCCTCCTACCTTTTCCCGGGGACCCCCAGCCCCTGC	-71
Human		G.....G.....A.....	
Simian		AGGGGCGGGGCTCCCCACCAA ACTAGCCCTGTTGCGCTCTCGGCAGTGCC	-19
Human		.....C..C.....	
Simian		GGGGGGCGCCGCCTCCCC	24
Human		.....	
Simian		Leu Pro Leu Leu Leu Pro Leu Leu Trp Leu Leu Val Leu	63
Human		CTG CCG CTG CTG CTA CCG CTG CTG TGG CTA CTG GTG CTG	
Simian		Thr Pro Gly Pro	102
Human		ACG CCT AGC CGG CCG GCC GCA GGA CTA TCC ACC TGC AAG	
Simian		Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu	141
Human		ACT ATC GAC ATG GAG CTG GTG AAG CGG AAG CGC ATC GAG	
Simian		Ala Thr Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala	180
Human		ACC ATC CGC GGC CAG ATC CTG TCC AAG CTG CGG CTC GCC	
Simian		Ser Pro Pro Ser Gln Gly Glu Val Pro Pro Gly Pro Leu	219
Human		AGC CCC CCG AGC CAG GGG GAG GTG CCG CCC GGC CCG CTG	
Simian		Pro Glu Ala Val Leu Ala Leu Tyr Asn Ser Thr Arg Asp	258
Human		CCC GAG GCC GTG CTC GCC CTG TAC AAC AGC ACC CGC GAC	

FIG. 1

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															1/7-1															
															90															
Simian	Arg	Val	Ala	Gly	Glu	Ser	Ala	Glu	Pro	Glu	Pro	Glu	Pro		297															
Human	CGG	GTG	GCC	GGG	GAG	AGT	GCG	GAG	CCG	GAG	CCC	GAA	CCG																	
	...	...	...	...	...	...	..A	..A	...	...	...	..G	..T																	
															110															
Simian	Glu	Ala	Asp	Tyr	Tyr	Ala	Lys	Glu	Val	Thr	Arg	Val	Leu		336															
Human	GAG	GCC	GAC	TAC	TAC	GCC	AAG	GAG	GTC	ACC	CGC	GTG	CTA																	
	...	...	...	...	...	...	...	...	...	...	...	...	...																	
															120															
Simian	Met	Val	Glu	Thr	His	Asn	Glu	Ile	Tyr	Asp	Lys	Phe	Lys		375															
Human	ATG	GTG	GAA	ACC	CAC	AAC	GAA	ATC	TAT	GAC	AAG	TTC	AAG																	
	...	...	...	...	...	...	...	...	...	...	...	...	...																	
															130															
Simian	Gln	Ser	Thr	His	Ser	Ile	Tyr	Met	Phe	Phe	Asn	Thr	Ser		414															
Human	CAG	AGC	ACA	CAC	AGC	ATA	TAT	ATG	TTC	TTC	AAC	ACA	TCA																	
	...	..T	...	...	...	...	...	...	...	...	...	...	...																	
															140															
Simian	Glu	Leu	Arg	Glu	Ala	Val	Pro	Glu	Pro	Val	Leu	Leu	Ser		453															
Human	GAG	CTC	CGA	GAA	GCA	GTA	CCT	GAA	CCT	GTG	TTG	CTC	TCC																	
	...	...	...	...	..G	...	...	...	..C	...	...	...	...																	
															160															
Simian	Arg	Ala	Glu	Leu	Arg	Leu	Leu	Arg	Leu	Lys	Leu	Lys		492																
Human	CGG	GCA	GAG	CTG	CGT	CTG	CTG	---	AGG	CTC	AAG	TTA	AAA																	
	...	...	...	...	...	...	...	AGG	...	...	...	...	...																	
															170															
Simian	Val	Glu	Gln	His	Val	Glu	Leu	Tyr	Gln	Lys	Tyr	Ser	Asn		531															
Human	GTC	GAG	CAG	CAT	GTG	GAG	CTG	TAC	CAG	AAA	TAC	AGC	AAC																	
	...	...	...	..C	...	...	...	...	...	...	...	...	...																	
															180															
Simian	Asn	Ser	Trp	Arg	Tyr	Leu	Ser	Asn	Arg	Leu	Leu	Ala	Pro		570															
Human	AAT	TCC	TGG	CGA	TAC	CTC	AGC	AAC	CGG	CTG	CTG	GCG	CCC																	
	...	...	...	...	...	...	...	...	...	...	...	..A	...																	
															200															
Simian	Ser	Asn	Ser	Pro	Glu	Trp	Leu	Ser	Phe	Asp	Val	Thr	Gly		609															
Human	AGC	AAC	TCG	CCG	GAG	TGG	TTG	TCT	TTT	GAT	GTC	ACC	GGA																	
	...	G..	...	..A	...	...	..A	...	...	...	...	...	...																	
															210															
Simian	Val	Val	Arg	Gln	Trp	Leu	Ser	Arg	Gly	Gly	Glu	Ile	Glu		648															
Human	GTT	GTG	CGG	CAG	TGG	TTG	AGC	CGC	GGA	GGG	GAA	ATT	GAG																	
	...	...	...	...	...	...	...	..T	...	...	...	...	...																	

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					220								Arg	
	Gly	Phe	Arg	Leu	Ser	Ala	His	Cys	Ser	Cys	Asp	Ser	Lys	
Simian	GGC	TTT	CGC	CTT	AGC	GCC	CAC	TGC	TCC	TGT	GAC	AGC	AAA	687
Human	...	...	...	...	...	...	...	...	...	...	...	...	.GG	
		230											240	
	Asp	Asn	Thr	Leu	Gln	Val	Asp	Ile	Asn	Gly	Phe	Thr	Thr	
Simian	GAT	AAC	ACA	CTG	CAA	GTG	GAC	ATC	AAC	GGG	TTC	ACT	ACC	726
Human	...	...	...	...	...	...	...	...	...	...	...	...	...	
								250						
	Gly	Arg	Arg	Gly	Asp	Leu	Ala	Thr	Ile	His	Gly	Met	Asn	
Simian	GGC	CGC	CGA	GGT	GAC	CTG	GCC	ACA	ATT	CAT	GGC	ATG	AAC	765
Human	...	...	...	...	...	...	...	..C	...	...	...	...	...	
						260								
	Arg	Pro	Phe	Leu	Leu	Leu	Met	Ala	Thr	Pro	Leu	Glu	Arg	
Simian	CGG	CCT	TTC	CTG	CTT	CTC	ATG	GCC	ACC	CCG	CTG	GAG	AGG	804
Human	...	...	...	...	...	...	...	...	...	...	...	...	...	
			270										280	
	Ala	Gln	His	Leu	Gln	Ser	Ser	Arg	His	Arg	Arg	Ala	Leu	
Simian	GCC	CAA	CAT	CTG	CAA	AGC	TCC	CGG	CAC	CGC	CGA	GCC	CTG	843
Human	...	..G	...	...	...	...	...	...	...	...	...	...	...	
										290				
	Asp	Thr	Asn	Tyr	Cys	Phe	Ser	Ser	Thr	Glu	Lys	Asn	Cys	
Simian	GAC	ACC	AAC	TAC	TGC	TTC	AGC	TCC	ACG	GAG	AAG	AAC	TGC	882
Human	...	...	...	..T	...	...	...	...	...	...	...	...	...	
														300
	Cys	Val	Arg	Gln	Leu	Tyr	Ile	Asp	Phe	Arg	Lys	Asp	Leu	
Simian	TGC	GTG	CGG	CAG	CTG	TAT	ATT	GAC	TTC	CGC	AAG	GAC	CTC	921
Human	...	...	...	...	...	..C	...	...	...	...	...	...	...	
														310
	Gly	Trp	Lys	Trp	Ile	His	Glu	Pro	Lys	Gly	Tyr	His	Ala	
Simian	GGC	TGG	AAG	TGG	ATC	CAC	GAG	CCC	AAG	GGC	TAC	CAT	GCC	960
Human	...	...	...	...	...	...	...	...	...	...	...	...	...	
														320
	Asn	Phe	Cys	Leu	Gly	Pro	Cys	Pro	Tyr	Ile	Trp	Ser	Leu	
Simian	AAC	TTC	TGC	CTG	GGG	CCC	TGT	CCC	TAC	ATT	TGG	AGC	CTG	999
Human	...	...	...	..C	...	...	..C	...	...	...	...	...	...	
														330
	Asp	Thr	Gln	Tyr	Ser	Lys	Val	Leu	Ala	Leu	Tyr	Asn	Gln	
Simian	GAC	ACG	CAG	TAC	AGC	AAG	GTC	CTG	GCC	CTG	TAC	AAC	CAG	1038
Human	...	...	...	...	...	...	...	...	...	...	...	...	...	

FIG. 1(cont.)

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	350														
Simian	His	Asn	Pro	Gly	Ala	Ser	Ala	Ala	Pro	Cys	Cys	Val	Pro	1077	
Human	CAT	AAC	CCG	GGC	GCC	TCG	GCG	GCG	CCG	TGC	TGC	GTG	CCG		
	...	...	...	...	...	...	...	...	...	...	...	...	...		
	360														
Simian	Gln	Ala	Leu	Glu	Pro	Leu	Pro	Ile	Val	Tyr	Tyr	Val	Gly	1116	
Human	CAG	GCG	CTG	GAG	CCA	CTG	CCC	ATC	GTG	TAC	TAC	GTG	GGC		
	...	...	...	...	..G	...	...	...	...	...	...	...	...		
	370														
Simian	Arg	Lys	Pro	Lys	Val	Glu	Gln	Leu	Ser	Asn	Met	Ile	Val	1155	
Human	CGC	AAG	CCC	AAG	GTG	GAG	CAG	CTG	TCC	AAC	ATG	ATC	GTG		
	...	...	...	...	...	...	...	...	...	...	...	...	...		
	380														
Simian	Arg	Ser	Cys	Lys	Cys	Ser								1199	
Human	CGC	TCC	TGC	AAA	TGC	AGC	TGA	GGCCCCGCCCCGCCCCGCCCCAC							
	...	...	...	..G	...	...	...	..T.....G.							
	390														
Simian	Arg	Ser	Cys	Lys	Cys	Ser								1199	
Human	CGC	TCC	TGC	AAA	TGC	AGC	TGA	GGCCCCGCCCCGCCCCGCCCCAC							
	...	...	...	..G	...	...	...	..T.....G.							
Simian	CCCGGCAGGCCCGGCCCGGCCCGGCCCGGCCCGGCTGTCTTGCCCTTGGG													1250	
Human	.....A.....G.....G.....C.....A.....														
Simian	GGCTGTATTTAAGGACACCCGTGCCCAAGCCACCTGGGGCCCCATTAAA														
Human	.....														
Simian	GA													1303	
Human	..														

FIG. 1(cont.)

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GCCCCCTCCCGTCAGTTCGCCAGCTGCCAGCCCCGGGACCTTTTCATCTCTTCCCTTTG															
GCCGGAGGAGCCGAGTTCAGATCCGCCACTCCGCACCCGAGACTGACACACTGAACTC															
CACTTCCTCCTCTTAAATTTATTTCTACTTAATAGCCACTCGTCTCTTTTTTTCCCCA															
TCTCATTGCTCCAAGAATTTTTTTCTTCTTACTCGCCAAAGTCAGGGTTCCCTCTGCC															
CGTCCCGTATTAATATTTCCACTTTTGGAAGTACTGGCCTTTTCTTTTAAAGGAATT															
CAAGCAGGATACGTTTTTCTGTTGGGCATTGACTAGATTGTTTGCAAAAGTTTCGCAT															
CAAAAACAACAACAACAAAAAACCAACAACCTCTCCTTGATCTATACTTTGAGAATTG															
TTGATTTCTTTTTTTTATTCTGACTTTTAAAAACAACCTTTTTTTTCCACTTTTTTAAA															
1 10															
Met His Tyr Cys Val Leu Ser Ala Phe Leu Ile Leu His Leu															
AA ATG CAC TAC TGT GTG CTG AGC GCT TTT CTG ATC CTG CAT CTG															
T															
20															
Val Thr Val Ala Leu Ser Leu Ser Thr Cys Ser Thr Leu Asp Met															
GTC ACG GTC GCG CTC AGC CTG TCT ACC TGC AGC ACA CTC GAT ATG															
30 40															
Asp Gln Phe Met Arg Lys Arg Ile Glu Ala Ile Arg Gly Gln Ile															
GAC CAG TTC ATG CGC AAG AGG ATC GAG GCG ATC CGC GGG CAG ATC															
50															
Leu Ser Lys Leu Lys Leu Thr Ser Pro Pro Glu Asp Tyr Pro Glu															
CTG AGC AAG CTG AAG CTC ACC AGT CCC CCA GAA GAC TAT CCT GAG															
60 70 *															
Pro Glu Glu Val Pro Pro Glu Val Ile Ser Ile Tyr Asn Ser Thr															
CCC GAG GAA GTC CCC CCG GAG GTG ATT TCC ATC TAC AAC AGC ACC															
80															
Arg Asp Leu Leu Gln Glu Lys Ala Ser Arg Arg Ala Ala Ala Cys															
AGG GAC TTG CTC CAG GAG AAG GCG AGC CGG AGG GCG GCC GCC TGC															
90 100															
Glu Arg Glu Arg Ser Asp Glu Glu Tyr Tyr Ala Lys Glu Val Tyr															
GAG CGC GAG AGG AGC GAC GAA GAG TAC TAC GCC AAG GAG GTT TAC															
110															
Lys Ile Asp Met Pro Pro Phe Phe Pro Ser Glu Thr Val Cys Pro															
AAA ATA GAC ATG CCG CCC TTC TTC CCC TCC GAA ACT GTC TGC CCA															
120 130															
Val Val Thr Thr Pro Ser Gly Ser Val Gly Ser Leu Cys Ser Arg															
GTT GTT ACA ACA CCC TCT GGC TCA GTG GGC AGC TTG TGC TCC AGA															

FIG. 2

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140										Ala	Ile	Pro	Pro	Thr			
Gln	Ser	Gln	Val	Leu	Cys	Gly	Tyr	Leu	Asp	GCC	ATC	CCG	CCC	ACT	447		
CAG	TCC	CAG	GTG	CTC	TGT	GGG	TAC	CTT	GAT								
150										160							
Phe	Tyr	Arg	Pro	Tyr	Phe	Arg	Ile	Val	Arg	Phe	Asp	Val	Ser	Ala	492		
<u>TTC</u>	TAC	AGA	CCC	TAC	TTC	AGA	ATT	GTT	CGA	TTT	GAC	GTC	TCA	GCA			
										G							
170										190							
Met	Glu	Lys	Asn	Ala	Ser	Asn	Leu	Val	Lys	Ala	Glu	Phe	Arg	Val	537		
ATG	GAG	AAG	AAT	GCT	TCC	AAT	TTG	GTG	AAA	GCA	GAG	TTC	AGA	GTC			
180										200							
Phe	Arg	Leu	Gln	Asn	Pro	Lys	Ala	Arg	Val	Pro	Glu	Gln	Arg	Ile	582		
TTT	CGT	TTG	CAG	AAC	CCA	AAA	GCC	AGA	GTG	CCT	GAA	CAA	CGG	ATT			
										T							
210										220							
Glu	Leu	Tyr	Gln	Ile	Leu	Lys	Ser	Lys	Asp	Leu	Thr	Ser	Pro	Thr	627		
GAG	CTA	TAT	CAG	ATT	CTC	AAG	TCC	AAA	GAT	TTA	ACA	TCT	CCA	ACC			
										G	C						
230										250							
Glu	Trp	Leu	Ser	Phe	Asp	Val	Thr	Asp	Ala	Val	His	Glu	Trp	Leu	717		
GAA	TGG	CTC	TCC	TTC	GAT	GTA	ACT	GAT	GCT	GTT	CAT	GAA	TGG	CTT			
										T							
240										260							
His	His	Lys	Asp	Arg	Asn	Leu	Gly	Phe	Lys	Ile	Ser	Leu	His	Cys	762		
CAC	CAT	AAA	GAC	AGG	AAC	CTG	GGA	TTT	AAA	ATA	AGC	TTA	CAC	TGT			
270										280							
Lys	Ser	Glu	Glu	Leu	Glu	Ala	Arg	Phe	Ala	Gly	Ile	Asp	Gly	Thr	852		
AAA	AGT	GAA	GAA	CTA	GAA	GCA	AGA	TTT	GCA	GGT	ATT	GAT	GGC	ACC			
										T							
290										310							
Ser	Thr	Tyr	Thr	Ser	Gly	Asp	Gln	Lys	Thr	Ile	Lys	Ser	Thr	Arg	897		
TCC	ACA	TAT	ACC	AGT	GGT	GAT	CAG	AAA	ACT	ATA	AAG	TCC	ACT	AGG			
300										310							
Lys	Lys	Asn	Ser	Gly	Lys	Thr	Pro	His	Leu	Leu	Leu	Met	Leu	Leu	942		
AAA	AAA	AAC	AGT	GGG	AAG	ACC	CCA	CAT	CTC	CTG	CTA	ATG	TTA	TTG			

FIG. 2(cont.)

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GCAAATTTTTTTTAAAGAAAAAATAAACACTGGAAGAATTTATTAGTGTTAATTATG	1641
TGAACAACGACAACAACAACAACAACAACAGGAAAATCCCATTAAGTGGAGTTG	1699
CTGTACGTACCGTTCCTATCCCGCGCCTCACTTGATTTTTCTGTATTGCTATGCAATA	1757
GGCACCCCTTCCCATTCTTACTCTTAGAGTTAACAGTGAGTTATTTATTGTGTGTTACT	1815
ATATAATGAACGTTTCATTGCCCTTGGAAAATAAAACAGGTGTATAAAGTGGAGACCA	1873
AATACTTTGCCAGAACTCATGGATGGCTTAAGGAAGTTGAACTCAAACGAGCCAGAA	1931
AAAAAGAGGTCATATTAATGGGATGAAAACCCAAGTGAGTTATTATATGACCGAGAAA	1989
GTCTGCATTAAGATAAAGACCCTGAAAACACATGTTATGTATCAGCTGCCTAAGGAAG	2047
CTTCTTGTAAGGTCCAAAACTAAAAAGACTGTTAATAAAAGAACTTTCAGT	2100
CAG(poly A)	2103

FIG. 2(cont.)

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-261	AGGGGATCTGTGGCAGGTCGGAGA---	AAGATC---CGTCTCCTGGGTACCAG	-215
ATCTCGCCCATCTAGGTTATTTC	CGTGGGATACTGAGACACCCCCGGTCCAAGCCTCC	-157	
CCTCCACCACTGCGCCCTTCTCCC	GTAAGGA-CCTCAA	CTTTCCCTCGAGGCCCTCCTA	-101
CCTTTTCCC	GGGGGACCCCCAGCCCCCTGCAGGGGCGGGGGCCTCCCCACCAA	ACTAGCC	-43
			1
CTGTT	CGCGCTCTCGGCAGTGCCGGGGGGGCGCCGCCTCCCCC	Met Pro Pro Ser ATG CCG CCC TCC	12
			10
Gly Leu Arg	Leu Leu Pro Leu Leu Leu Pro Leu Leu Trp Leu Leu	Tyr Leu Leu	57
GGG CTG CGG	CTG CTG CCG CTG CTG CTA CCG CTG CTG TGG CTA CTG		
			20
Val Leu Thr	Pro Ser Arg Pro Ala Ala Gly Leu Ser Thr Cys Lys		102
GTG CTG ACG	CCT AGC CGG CCG GCC GCA GGA CTA TCC ACC TGC AAG		
			40
Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg Ile Glu Thr Ile	ACT ATC GAC ATG GAG CTG GTG AAG CGG AAG CGC ATC GAG ACC ATC		147
			50
Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala Ser Pro Pro Ser	CGC GGC CAG ATC CTG TCC AAG CTG CGG CTC GCC AGC CCC CCG AGC		192
			70
Gln Gly Glu Val Pro Pro Gly Pro Leu Pro Glu Ala Val Leu Ala	CAG GGG GAG GTG CCG CCC GGC CCG CTG CCC GAG GCC GTG CTC GCC		237
			80
Leu Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly Glu Ser Ala Glu	CTG TAC AAC AGC ACC CGC GAC CGG GTG GCC GGG GAG AGT GCG GAG		282
			100
Pro Glu Pro Glu Pro Glu Ala Asp Tyr Tyr Ala Lys Glu Val Thr	CCG GAG CCC GAA CCG GAG GCC GAC TAC TAC GCC AAG GAG GTC ACC		327
			110
Arg Val Leu Met Val Glu Thr His Asn Glu Ile Tyr Asp Lys Phe	CGC GTG CTA ATG GTG GAA ACC CAC AAC GAA ATC TAT GAC AAG TTC		372
			130
Lys Gln Ser Thr His Ser Ile Tyr Met Phe Phe Asn Thr Ser Glu	AAG CAG AGC ACA CAC AGC ATA TAT ATG TTC TTC AAC ACA TCA GAG		417
			140
Leu Arg Glu Ala Val Pro Glu Pro Val Leu Leu Ser Arg Ala Glu	CTC CGA GAA GCA GTA CCT GAA CCT GTG TTG CTC TCC CGG GCA GAG		462

FIG. 3

Leu	Arg	Leu	Leu		160	Arg	Leu	Lys	Leu	Lys	Val	Glu	Gln	His	Val	504
CTG	CGT	CTG	CTG	—	AGG	CTC	AAG	TTA	AAA	GTC	GAG	CAG	CAT	GTG		
170										180						
Glu	Leu	Tyr	Gln	Lys	Tyr	Ser	Asn	Asn	Ser	Trp	Arg	Tyr	Leu	Ser		549
GAG	CTG	TAC	CAG	AAA	TAC	AGC	AAC	AAT	TCC	TGG	CGA	TAC	CTC	AGC		
					190											
Asn	Arg	Leu	Leu	Ala	Pro	Ser	Asn	Ser	Pro	Glu	Trp	Leu	Ser	Phe		594
AAC	CGG	CTG	CTG	GCG	CCC	AGC	AAC	TCG	CCG	GAG	TGG	TTG	TCT	TTT		
200										210						
Asp	Val	Thr	Gly	Val	Val	Arg	Gln	Trp	Leu	Ser	Arg	Gly	Gly	Glu		639
GAT	GTC	ACC	GGA	GTT	GTG	CGG	CAG	TGG	TTG	AGC	CGC	GGA	GGG	GAA		
					220											
Ile	Glu	Gly	Phe	Arg	Leu	Ser	Ala	His	Cys	Ser	Cys	Asp	Ser	Lys		684
ATT	GAG	GGC	TTT	CGC	CTT	AGC	GCC	CAC	TGC	TCC	TGT	GAC	AGC	AAA		
230										240						
Asp	Asn	Thr	Leu	Gln	Val	Asp	Ile	Asn	Gly	Phe	Thr	Thr	Gly	Arg		729
GAT	AAC	ACA	CTG	CAA	GTG	GAC	ATC	AAC	GGG	TTC	ACT	ACC	GGC	CGC		
					250											
Arg	Gly	Asp	Leu	Ala	Thr	Ile	His	Gly	Met	Asn	Arg	Pro	Phe	Leu		774
CGA	GGT	GAC	CTG	GCC	ACA	ATT	CAT	GGC	ATG	AAC	CGG	CCT	TTC	CTG		
260										270						
Leu	Leu	Met	His	Thr	Pro	Leu	Glu	Arg	Ala	Gln	His	Leu	Gln	Ser		819
CTT	CTC	ATG	GCC	ACC	CCG	CTG	GAG	AGG	GCC	CAA	CAT	CTG	CAA	AGC		
					280											
Ser	Arg	His	Arg	Arg	Ala	Leu	Asp	Ala	Ala	Tyr	Cys	Phe	Arg	Asn		864
TCC	CGG	CAC	CGC	CGA	GCT	TTG	GAT	GCG	GCC	TAT	TGC	TTT	AGA	AAT		
290										300						
Val	Gln	Asp	Asn	Cys	Cys	Leu	Arg	Pro	Leu	Tyr	Ile	Asp	Phe	Lys		909
GTG	CAG	GAT	AAT	TGC	TGC	CTA	CGT	CCA	CTT	TAC	ATT	GAT	TTC	AAG		
					310											
Arg	Asp	Leu	Gly	Trp	Lys	Trp	Ile	His	Glu	Pro	Lys	Gly	Tyr	Asn		954
AGG	GAT	CTA	GGG	TGG	AAA	TGG	ATA	CAC	GAA	CCC	AAA	GGG	TAC	AAT		
320										330						
Ala	Asn	Phe	Cys	Ala	Gly	Ala	Cys	Pro	Tyr	Leu	Trp	Ser	Ser	Asp		999
GCC	AAC	TTC	TGT	GCT	GGA	GCA	TGC	CCG	TAT	TTA	TGG	AGT	TCA	GAC		
					340											
Thr	Gln	His	Ser	Arg	Val	Leu	Ser	Leu	Tyr	Asn	Thr	Ile	Asn	Pro		1044
ACT	CAG	CAC	AGC	AGG	GTC											

# SUBSTITUTE SHEET

7/7-1															
350											360				
Glu	Ala	Ser	Ala	Ser	Pro	Cys	Cys	Val	Ser	Gln	Asp	Leu	Glu	Pro	
GAA	GCA	TCT	GCT	TCT	CCT	TGC	TGC	GTG	TCC	CAA	GAT	TTA	GAA	CCT	1089
C															
370															
Leu	Thr	Ile	Leu	Tyr	Tyr	Ile	Gly	Lys	Thr	Pro	Lys	Ile	Glu	Gln	
CTA	ACC	ATT	CTC	TAC	TAC	ATT	GGC	AAA	ACA	CCC	AAG	ATT	GAA	CAG	1134
380											390				
Leu	Ser	Asn	Met	Ile	Val	Lys	Ser	Cys	Lys	Cys	Ser	***			
CTT	TCT	AAT	ATG	ATT	GTA	AAG	TCT	TGC	AAA	TGC	AGC	TAA	AATTCT		1179
TGGAAAAGTGGCAAGACCAAAATGACAATGATGATGATAATGATGATGACGACGACAA															
1237															
CGATGATGCTTGTAACAAGAAAACATAAGAGAGCCTTGGTTCATCAGTGTTAAAAAAT															
1295															
TTTTGAAAAGGCGGTACTAGTTCAGACACTTTGGAAGTTTGTGTTCTGTTTGTTAAAA															
1353															
CTGGCATCTGACACAAAAAAGTTGAAGGCCTTATTCTACATTTACCTACTTTGTAA															
1411															
GTGAGAGAGACAAGAAGCAAATTTTTTTTAAAGAAAAAATAAACACTGGAAGAATTT															
1469															
ATTAGTGTTAATTATGTGAACAACGACAACAACAACAACAACAACAGGAAAATC															
1527															
CCATTAAGTGGAGTTGCTGTACGTACCGTTCCTATCCCGCGCCTCACTTGATTTTTCT															
1585															
GTATTGCTATGCAATAGGCACCCTTCCCATTCTTACTCTTAGAGTTAACAGTGAGTTA															
1643															
TTTATTGTGTGTTACTATATAATGAACGTTTCATTGCCCTTGGAAAATAAAACAGGTG															
1701															
TATAAAGTGGAGACCAAATACTTTGCCAGAACTCATGGATGGCTTAAGGAACTTGAA															
1759															
CTCAAACGAGCCAGAAAAAAGAGGTCATATTAATGGGATGAAAACCCAAGTGAGTTA															
1817															
TTATATGACCGAGAAAGTCTGCATTAAGATAAAGACCCTGAAAACACATGTTATGTAT															
1875															
CAGCTGCCTAAGGAAGCTTCTTGTAAGGTCCAAAACTAAAAAGACTGTTAATAAAAG															
1933															
AAACTTTCAGTCAG(poly A)															
1947															

FIG. 3(cont.)

# INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US91/04449**

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all) \*

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/36  
US.CL.: 514/8

**II. FIELDS SEARCHED**

Minimum Documentation Searched ?

Classification System	Classification Symbols
U.S.	514/2,8,12,21; 424/85.1, 88; 530/380,395,399

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched \*

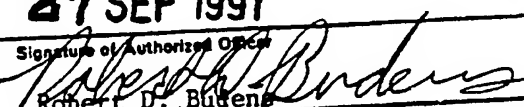
Databases: Dialog (Files 5, 73, 155, 351); USPTO Automated Patent System (File USPAT, 1971-1991).

III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
Y	NATURE, Vol. 316, issued 22 August 1985, Derynck et al., "Human transforming Growth Factor- $\beta$ Complementary DNA Sequence and Expression in Normal and Transformed Cells," pages 701-705, see entire document.	1-2,4-5, 7-8,10-11 13-14,16-17
Y	Cell, Vol. 49, issued 22 May 1987, Massague, TGF- $\beta$ Family of Growth and Differentiation Factors," pages 437-438, see entire document.	1-2,4-11, 13-18
Y	DNA, Vol. 7, NO. 1, issued 1988, Madisen et al., "Transforming Growth Factor- $\beta$ 2: cDNA Cloning and Sequence Analysis," pages 1-8, see entire document.	1,4,6,7, 9-10,13 15-16,18.
Y	DNA, Vol. 7, No. 7, issued 1988, Webber et al., "Structural and Sequence Analysis of TGF- $\beta$ 2 cDNA Clones Predicts Two Different Precursor Proteins Produced by Alternative mRNA splicing," pages 493-497, see entire document.	1,6,7,10 15,16,18

- \* Special categories of cited documents: 10
- "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier document but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "A" document member of the same patent family

**IV. CERTIFICATION**

Date of the Actual Completion of the International Search  <b>11 September 1991</b> International Searching Authority  <b>ISA/US</b>	Date of Mailing of this International Search Report <b>27 SEP 1991</b> Signature of Authorized Officer  <b>Robert D. Budenz</b>
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Cell, Vol. 48, issued 13 February 1987, Cheifetz et al., "The Transforming Growth Factor-B System, a Complex Pattern of Cross-Reactive Ligands and Receptores", pages 409-415, see entire document.	1,4,7,10,13,16



## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE<sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers \_\_\_\_\_, because they relate to subject matter<sup>12</sup> not required to be searched by this Authority, namely:

2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out<sup>13</sup>, specifically:

3. ☐ Claim numbers \_\_\_\_\_, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING<sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

SEE ATTACHED SHEET

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only these claims of the international application for which fees were paid, specifically claims: 1-2, 4-11, 13-18

## TELEPHONE PRACTICE

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

In the examination of international applications filed under the Patent Cooperation Treaty, PCT Rule 13.1 states that the international application shall relate to one invention only or to a group of inventions so linked as to form "a single general inventive concept."

PCT Rule 13.2 indicates that this shall be construed as permitting, in particular, one of the following three possible combinations of the claimed invention:

- (1) a product, a process specifically adapted for the manufacture of said product and a use of said product, or
- (2) a process, and an apparatus or means specifically designed for carrying out said process, or
- (3) a product, a process specially adapted for the manufacture of said product and an apparatus or means designed for carrying out the process.

Additionally, current United States Patent and Trademark Office restriction practice permits the following combinations of the claimed invention:

- (4) a product, and a process specifically adapted for the manufacture of said product, and
- (5) a product, and a use of the said product, as where said use as claimed cannot be practiced with another materially different product.

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-18, a first method drawn to methods of treating hypertension.

Group II, claims 19-36, a second method drawn to methods of treating hypotension.

Group III, a first specie of TGF- $\beta$  drawn to TGF- $\beta$ 1.

Group IV, a second specie of TGF- $\beta$  drawn to TGF- $\beta$ 2.

Group V, a third specie of TGF- $\beta$  drawn to TGF- $\beta$ 1/ $\beta$ 2 hybrids.

Group VI, a fourth specie of TGF- $\beta$  drawn to TGF- $\beta$ 1 precursor.

Group VII, a fifth specie of TGF- $\beta$  drawn to TGF- $\beta$ 2 precursor.

Group VIII, a sixth specie of TGF- $\beta$  drawn to TGF- $\beta$ 1/TGF- $\beta$ 2 precursor.

Group IX, a seventh specie of TGF- $\beta$  drawn to TGF- $\beta$ 1 complex.

Group X, an eighth specie of TGF- $\beta$  drawn to TGF- $\beta$ 2 complex.

The inventions listed as Groups I-X do not meet the requirements for Unity of Invention for the following reasons:

The inventions of Groups I and II are directed to methods of treating two pathologic disease states using different reagents and are not so linked as to form a single general inventive concept.

The inventions of Groups III-X are directed to species of TGF- $\beta$  that differ in physical properties such as chemical composition, primary amino acid sequence and molecular weight, and are not so linked as to form a single general inventive concept.

During a telephonic requirement for election, on August 8, 1991, applicant's representative, Brian W. Poor, elected the invention of Groups I, III, and the additional Groups V-X for examination.

